

Applicants: Deiss, et al.
Serial No. 09/499,553
Filed: February 7, 2000
Page 2

phenotype comprising the steps of:

- B1
Cont
- a) identifying a cell type with a phenotype of interest;
 - b) inactivating genes in the cell of interest with a gene inactivation means on an aliquot of a culture of the cell type;
 - c) applying selection means to an aliquot of the cell culture of step (b) and reserving an aliquot of unselected cells;
 - d) isolating the selected cells of step (c);
 - e) isolating the vectors from the selected cells and from the control unselected aliquot of cells;
 - f) isolating the gene inactivating elements from the vectors; and
 - g) utilizing differential analysis means to identify the genes inactivated in the cells in step (c) that affect the phenotype of interest.

Applicants: Deiss, et al.
Serial No. 09/499,553
Filed: February 7, 2000
Page 3

²⁹
21. (New) The method as set forth in claim ²⁸20 wherein said gene inactivation means are performed using a Technical Knock Out (TKO) inactivation.

³⁰
22. (New) The method as set forth in claim ²⁸20 wherein the phenotype of interest can be selected from the group consisting of phenotype relating to growth, phenotype relating to release of factors, phenotype relating to expression of factors and phenotype relating to cell function.

³¹
23. (New) The method as set forth in claim ²⁸20 wherein the selection means can be selected from the group consisting of ability of cells to survive under specific culture conditions, ability of cells to express a specific measurable factors, detectable changes in cell structure, and differential gene expression.

³²
24. (New) The method as set forth in claim ²⁸20 wherein differential analysis means are selected from the group of methods consisting of differential display, representational differential analysis (RDA), suppressive subtraction hybridization (SSH), serial analysis of gene expression (SAGE), gene expression microarrays, nucleic acid chip technology, and direct sequencing.

Applicants: Deiss, et al.
Serial No. 09/499,553
Filed: February 7, 2000
Page 4

³³
~~25~~. (New) A method for the identification of genes that are essential for the maintenance of specific cell phenotypes including the steps of:

- Rule 126*
- a) identifying a cell type with a phenotype of interest;
 - b) preparing an expression cDNA library from cells expressing the phenotype;
 - B1*
Cont c) transfecting a cell culture of the cell type with expression vectors incorporating the expression cDNA library;
 - d) applying selection means to an aliquot of the transfected cell culture in step (c) and reserving an untreated aliquot;
 - e) isolating cells which continue to maintain the phenotype and isolating the expression vector, or the DNA insert found in the expression vector, from the cells maintaining the phenotype;
 - f) isolating the expression vector, or the DNA insert found in the expression vector from the reserved untreated aliquot of the cells; and

Applicants: Deiss, et al.
Serial No. 09/499,553
Filed: February 7, 2000
Page 5

g) identifying expression vectors, or the DNA insert found in the expression vector, that are present in the reserved aliquot and not in cells maintaining the phenotype by subtraction means whereby expression vectors are identified that have targeted genes that affect the phenotype.

Rule 126
B1
cond
³⁴
³³
²⁶. (New) The method as set forth in claim ²⁵ wherein the step of recloning and sequencing the expression vectors that target the genes that affect the phenotype is performed on the identified antisense expression vectors.

³⁵
²⁷. (New) The method of claim ²⁵ wherein the expression vector comprises a cDNA which is found in antisense orientation and which expresses antisense RNA.

³⁶
²⁸. (New) The method of claim ²⁵ wherein the expression vector comprises dominant negative gene fragments.

REMARKS

Claims 1-19 were pending in the subject application. By this Amendment applicants have canceled claims 1-19 and added new claims 20-28. Accordingly, claims 20-28 are currently pending and under examination.